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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Takashi FUJITA et al.

Serial No. : 09/991,100

Filed : November 21, 2001

For : THIAZOLIDINE-2,4-DIONE HYDROCHLORIDE SALT, PHARMACEUTICAL COMPOSITIONS THEREOF AND TREATMENT METHOD THEREWITH

Art Unit : 1626

Examiner : Laura Lynne Stockton

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Attorney: MARSHALL J. CHICK

Dated: May 14, 2003

In the event that this Paper is late filed, and the necessary petition for extension of time is not filed concurrently herewith, please consider this as a Petition for the requisite extension of time, and to the extent not tendered by check attached hereto, authorization to charge the extension fee, or any other fee required in connection with this Paper, to Account No. 06-1378.

TRANSMITTAL OF CORRECTED DECLARATION (UNEXECUTED)

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S I R :

Along with the AMENDMENT filed May 7, 2003, we filed an unexecuted DECLARATION providing evidence in support of the arguments presented therein. We received some additional information from the applicants explaining that the data with respect to the solubility testing was accomplished under the supervision and control of Dr. Tomonori KONSE. The DECLARATION was, therefore, augmented to include this information (and to correct some minor spelling errors). It is expected that an

executed version will be available shortly to complete the record. However, an unexecuted version is being filed concurrently herewith to provide early notice of the change. The data remains unchanged.

Respectfully submitted,

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Attorney Docket No. 01626C/HG

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Serial No.	:	09/991,100
Filed	:	November 21, 2001
For	:	THIAZOLIDINE-2,4-DIONE HYDROCHLORIDE SALT, PHARMACEUTICAL COMPOSITIONS THEREOF AND TREATMENT METHOD THEREWITH
Art Unit	:	1626
Examiner	:	Laura Lynne Stockton

SECOND DECLARATION UNDER 37 CFR 1.132

Dr. Kazushi ARAKI declares that he obtained his D.V.M. degree in Veterinary medicine from Tokyo University of Agriculture and Technology in 1992 and obtained his Ph.D. in 1996 from Tokyo University. His Ph.D. research was Meiotic abnormalities of c-mos knockout mouse oocytes : activation after first meiosis or entrance into third meiotic metaphase.

He joined Sankyo in 1996. He is now an Associate Chief Researcher in Pharmacology & Molecular Biology res. labs. in Sankyo. His research interests are antidiabetic mechanism of insulin sensitizer in diabetic animal model; characterization of diabetic animal models and pharmacology of antidiabetic drugs.

Dr. Tomonori KONSE declares that he obtained his Ph.D. in 1988, 'Electrochemical study about the redox properties of

anthracycline antibiotics, Adriamycin', from Figu Pharmaceutical University; from 1997 to 1998 he studied at the Chemistry Department in Indiana University (Prof. M.V. Novotny) as an Invited Researcher, for "Study of the analytical method of glycoproteins'.

He joined Sankyo in 1988 and now is Group Director, in Product Development Laboratories in Sankyo. His research interest is in the development and validation of analytical method for drug substance and drug product and the establishment of the specifications and analytical method for the application of medicine.

In order to show that the activity of the claimed salt form of the compound when used in the claimed method provides surprisingly superior results, additional comparative data was obtained under their supervision and control. Of the following, Dr. ARAKI supervised Test 2 and provided the primary discussion. Dr. KONSE supervised Test 1 solubility test and the discussion thereof.

As shown below, two kinds of additional data are presented in order to explain the invention's effect more clearly. Thereafter, there is a discussion of the additional data together with the prior data, which was shown in this application and the previous Declaration. Finally, the

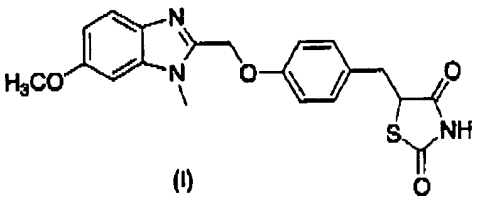
expectations of the art, based on the art of record, are established.

Test 1

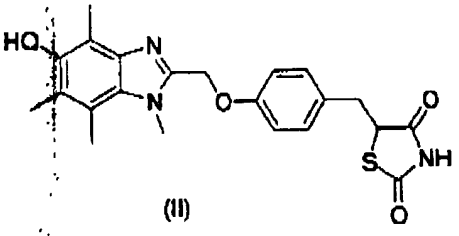
Solubility test

Test compounds

Two compounds which have a structure of formula (II) are used.



(I)



(II)

In the case of the compounds of formula (II), its hydrochloride salt and free form are called Compound C and Compound D respectively. Compound C and D are from the cited Fujita patent and these are disclosed with physical properties. (Compound C is Example 4 and Compound D is Example 3 in the patent.)

In the case of the compounds of formula (I), its hydrochloride salt and free form are called Compound A and Compound B respectively. Compound A is the hydrochloride salt claimed in this application.

One of the reasons why Compound C and D were chosen is that Compound C is the only hydrochloride compound prepared in the Fujita patent. In addition, the structure of those compounds is similar to that of Compound A. For instance the structural difference of the comparative Compound C and D from Compound A is only at the substituent groups on the benzimidazole moiety.

Above mentioned 4 compounds (compound A, B, C and D) are the same compounds' definition as those in the previous Declaration.

Test Methods

Solubility test have been done. This test method is same as described in the present application.

Test Example

To 100 ml of the 1st fluid of Japanese Pharmacopeia (1000 ml solution made by mixing 2.0 g of sodium chloride with 7.0 ml of hydrochloric acid and water) were added 25 mg of Compound C or Compound D, and the mixture was stirred with a stirrer at 37°C in a 200 ml Erlenmeyer flask. One hour later, 10 ml of the sample were filtered through Acrodisk LC 13 (PVDF, manufactured by German Science Co.). The initial 3 ml were discarded, and the following 7 ml were taken into a test tube. Of this sample, 5 ml were taken accurately with a whole

pipette and added to 2 ml of methanol accurately measured in advance in a test tube.

The quantity was measured by HPLC and the solubility was decided from a calibration curve made according to the following procedure.

The calibration curve was made by preparing a methanolic standard solution of Compound D at a concentration of 200 µg/ml, 40 µg/ml and 10 µg/ml, mixing each 2 ml of the standard solution with 5 ml of the 1st fluid of Japanese Pharmacopeia and determined by HPLC.

HPLC Condition:

Analytical column: L-column ODS (4.6 mm IDx15 cm, manufactured by Chemicals Evaluation Research Institute Japan)

Mobile phase: 0.01 mol/L acetic acid buffer solution (pH 5.0)/acetonitrile mixture (13: 7) Flow rate: about 1.0 ml/min.

Column temperature: 40°C.

Detector: ultraviolet absorptiometer (measuring wavelength: 290 nm)

Comparative Test Results

The comparative test data are as shown below.

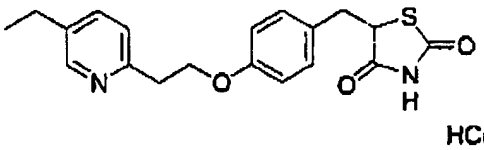
Table 1

	0 Hour	Solubility 1.0 hour later (µg/mL)
Compound D	0	46.4
Compound C	0	148

Test 2

Relationship between plasma glucose lowering rate and plasma glucose.

Test compounds



Pioglitazone hydrochloride

Pioglitazone is the compound of Example 1 in USP No. 4,687,777.

As shown above, pioglitazone hydrochloride is used as a test compound. Pioglitazone hydrochloride is a therapeutic agent for diabetes named "Actos" from Takeda Chemical Industries, Ltd. One of the reasons why it was chosen is that it has the same glucose lowering mechanism as compound A. And the other reason is that this compound has the same specific structure of benzylthiazolidinedione skeleton, as that of Compound A.

Test Methods

Hypoglycemic test and Plasma concentration test have been done.

Test Example

1. Hypoglycemic action

On day 0, the blood sample was collected from the tail vein of each KK mouse (4-5 month old) which developed diabetes

and the plasma glucose level was measured after centrifugation. These mice were then classified into 7 groups (6 mice per group). For seven days, powdered food (F-2, Funabashi Farm) which contains the test compound at adjusted concentrations of 0.0001%, 0.0003%, 0.001%, 0.003%, 0.01%, and 0.03% (pioglitazone hydrochloride) was given to mice. The mouse group given the test compound refers to "the medicine administered group", and that given the powdered food containing free of the test compound refers to "the control". On day 7, the blood sample was collected from the tail vein of each mouse and the plasma glucose level was measured by a glucose analyzer ("Glucolcader-GXT", A&T Inc.). The plasma glucose lowering rate was calculated using the following equation:

Plasma glucose lowering rate (%)=

(average plasma glucose level of the control - average plasma glucose level of the medicine administered group) X 100 / the plasma glucose level of the control

At the end of experiment, the mice were killed by decapitation and the blood samples were collected for measurement of plasma pioglitazone concentration.

2. Analytical methods for the measurement of plasma concentration

1) Preparation of analytical samples from plasma samples

The plasma sample (30 µl) was mixed well with 30 µl of the internal standard (I.S.) solution containing a proper compound and 60 µl of methanol in 1.5 ml plastic tube and centrifuged at 15,000 rpm for 10 min at 4°C. The supernatant obtained was used for measurement of pioglitazone by HPLC.

2) Samples for calibration curve and quality control (QC)

The control plasma (30 µl) was mixed well with 30 µl of the standard solution, 30 µl of the I.S. solution and 30 µl of methanol in 1.5 ml plastic tube. The samples were processed as described above. The concentrations of the calibration standard samples were 0.0391, 0.0781, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00, 10.0 and 20.0 µg/ml as pioglitazone concentrations.

The concentrations of the QC samples were 0.0781, 1.25 and 20.0 µg/ml as pioglitazone concentrations.

3) HPLC analysis

The conditions for HPLC analysis were as follows.

HPLC apparatus: LC-10Avp (Shimazu Corp.)

Column: YMC-Pack ODS-A-312 (150x6.0 mm ID, S-5 mm, YMC Co., Ltd.)

Column temperature: 35°C

Flow rate: 1.0 ml/min

UV detection: 225 nm

Mobile phase: CH_3CN: H_2O: Triethylamine: CH_3COOH = 35:65:0.1:0.1 (v/v)

Injection volume: 30 µl

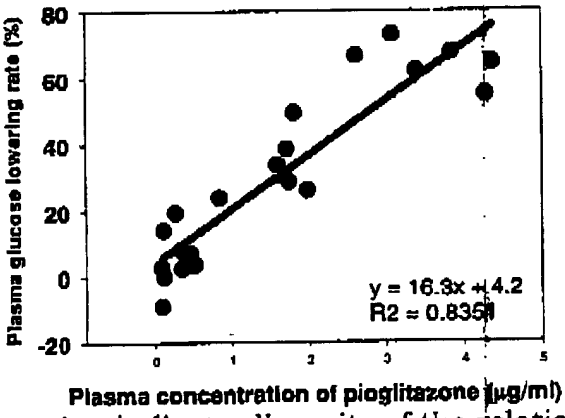
4) Standard curve for pioglitazone in plasma

Standard curves of the nominal concentration against the peak areas of pioglitazone to the I.S. were fitted by the least-squares linear regression method (y = ax+b, weight: 1 /y2) using the computer software Millennium32 (Nihon Waters K.K.). The limit of quantitation was defined as the minimum concentration having accuracy within ± 20%. The QC samples, prepared in duplicate at each of three concentration of pioglitazone described above were used to evaluate intra-assay reproducibly. The plasma concentrations of pioglitazone were calculated using the above standard curve.

Test Results

The relationship between plasma concentration and plasma glucose lowering rate is as shown below.

Graph 1



(R2 value indicates linearity of the relationship. When the value is closer to 1, the linearity is higher.)

Discussion

In order to discuss the above data together with the prior data, the solubility data and the glucose lowering rate data are collected together and presented below.

Solubility test results

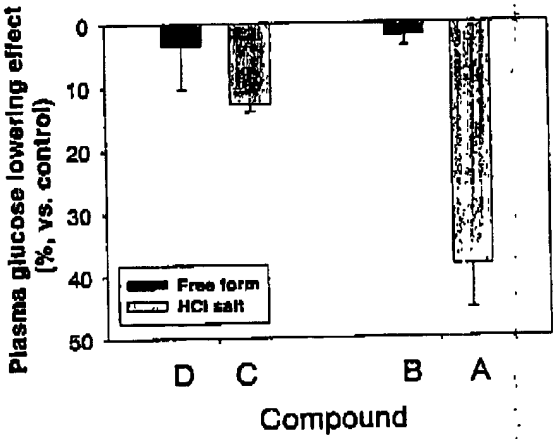
Table 2

	0 Hour	Solubility 1.0 hour later (μg/mL)
Compound B	0	41.0
Compound A	0	86.4
Compound D	0	46.4
Compound C	0	148

(Data of compound A and B are quoted from Table 2 of instant application. Data of compound C and D are quoted from Table 1 of this argument.)

Hypoglycemic activity test results

Graph 2



(Graph 2 is quoted from the previous Declaration.)

From Table 2, both compounds show the solubility increasing effect by salt forming, however, all 4 compounds

still show poor solubility. So that the compounds, which show solubility rate like Table 2, are classified as the poor soluble compound. Because a series of compounds, which have benzylthiazolidine skeleton, show the same tendency of solubility, all of them would be classified as the poor soluble compounds.

Regarding series of poor soluble compounds, Shargel et al. (Encyclopedia of Pharmaceutical Technology Second Edition Vol.1, p. 156-176, 2002) on page 167, columns 1, states, "In biologic systems, drug dissolution in an aqueous medium is an important prior condition of systemic absorption. The rate at which drugs with poor aqueous solubility dissolve from an intact or disintegrated solid dosage form in the GI tract often controls the rate of systemic absorption of the drug." In other words, the solubility of poor soluble compounds have a correlation between the solubility and the blood concentration. Therefore when solubility of the compound increases by salt forming, blood concentration of it should also increase (see enclosed copy).

Moreover it is well known that a series of compounds, which posses a same specific skeleton and a same bioactivity mechanism, has same correlation between its blood concentration and its bioactivity rate. Graph 1 shows almost linear relationship between blood concentration and plasma glucose lowering rate according to its R2 value. Therefore,

this data also supports the expectation that in the series of these compounds that there is a linear correlation between their plasma glucose lowering rates and their blood concentrations.

In the case of C and D in Table 1, there is a solubility data of compound C that shows nearly 3 times higher than its free form compound D. According to the relationship between blood concentration and solubility as mentioned above, the improving effect of blood concentration, which is expected from the solubility result, should be 3 times. Then at plasma glucose lowering rate of Graph 2, the improving effect due to salt forming is approximately 3 times. Because C and D exist as the same free form D in the blood, this data also support the expectation of a linear correlation between blood concentration and plasma glucose lowering rate of these compounds.

Based on the above, it is submitted that one skilled in the art at the time of invention would expect that solubility and plasma glucose lowering rate would correlate in the case of the compounds, which have benzylthiazolidine skeleton and insulin sensitizing action. This would lead to the expectation from the solubility data of A (Table 2) that there would be and improving effect of 2 times by salt forming: Because A and B exist as the same freeform B in the blood, if

solubility becomes 2 times higher, the bioactivity should become 2 times stronger.

By contrast, the activity of compound A is about twenty times stronger than that of its free form compound B at the plasma glucose lowering rate (Graph 2). It is submitted that, from the above mentioned reference and common knowledge, this salt forming effect of hydrochloride salt is far stronger than that which could have been expected. Therefore one skilled in the art at the time of the invention would not have expected this specific improving effect of the salt.

Further to above information, Berge et al. teach that it could be generally expected to prepare the salt of known compounds with increasing physicochemical properties such as solubility and hygroscopicity. Moreover Berge et al. on page 16, columns 1, states, "At present, selecting a salt form that exhibits the desired combination of properties is a difficult semiempirical choice." Therefore, as described in the previous Declaration, Berge et al. does not teach or suggest concrete improving activity effect that would be expected to prepare hydrochloride salt of the known compounds such as the compounds within the scope of the reference patent.

In view of above data and knowledge, the salt forming effect of instant claimed hydrochloride salt is far stronger than that would have been expected for this kind of compounds.

Therefore one skilled in the art at the time of the invention would not have expected this specific effect of the salt.

In conclusion, Compound A shows an unexpected bioactivity improving effect compared to the compounds within the broad scope of the reference patent.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:_____ _____
Kazushi ARAKI

Date:_____ _____
Tomonori KONSE

Enc. Encyclopedia of Pharmaceutical Technology Second Edition Vol.1, p.156-176, 2002